UV AND TEMPERATURE TEST

Why to do this :

1. Determine the best way to kill bacteria

What you need :

1. Culture media : LB

- 10 g bactotrypton
- 5 g yeast extract
- 5 g NaCl
- 0,5 mL NaOH 10N
- Qsp 1 L

2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL
Tetracycline (Tet) : 1 mg/mL
Kanamycin (Kann) : 5 mg/mL
Ampicillin (Amp) : 10 mg/mL

⟶ 50 µL antibiotic / 5mL medium

3. Apparatus : UV exposure, PCR, microscope.

How to do :

Be aware of being in sterile conditions!

1. Spots on LB plates to count survival bacteria

a) Prepare 5 dilutions from the culture exposed to UV/temperature and the control:
   - 100 times : put 10µl culture into 990µl sterile water
   - 10^4 times : put 10µl previous culture into 990µl sterile water
   - 10^5 times : put 20µl previous culture into 180µl sterile water
   - 10^6 times : put 20µl previous culture into 180µl sterile water

b) Drop off 10 µl of each concentration on LB plates (containing antibiotic appropriate with the strain) for the culture exposed to UV/temperature and the control.

c) Incubate plates at 37°C, when drops start drying

2. Extraction of genomic DNA from concentrated cultures

d) Preheat a bath at 99°C

e) Centrifuge 800 µl liquid cultures

f) Centrifuge 800 µl liquid cultures (in micro tubes) 5 minutes at 12000 rpm and discard half of the supernatant (to concentrate bacteria). Resuspend by pipetting up and down.

g) Put it at 99°C for 5 min then vortex a little bit or pipette up and down. Repeat this operation.

h) Centrifuge (in micro tubes) 5 minutes at 14000 g

i) Take 20µl supernatant and add 4µl Buffer stop. Run an electrophoresis gel

3. PCR

a) Prepare in a micro tube a premix composed with

- Water	16,25 µl
- Primer Forward (FW)	1 µl
- Primer Reverse (RV)	1 µl
- Deoxy-nucleotide-triphosphate (dNTP)	0,5 µl
- Enzyme Q5 Buffer	5 µl
- Enzyme Q5	0,25 µl

b) The volume indicated must be multiply by n+1 where n is the number of samples (to be sure to pipette the same volume for each).

c) In case of a culture being exposed to UV/temperature and its control, n would be equal to 3, leading to the following quantities:

- Water	48,75 µl
- Primer Forward (FW)	3 µl
- Primer Reverse (RV)	3 µl
- Deoxy-nucleotide-triphosphate (dNTP)	1,5 µl
- Enzyme Q5 Buffer	15 µl
- Enzyme Q5	0,75 µl

Be aware to put premix micro tube in ice after adding Q5 enzyme !

d) For each sample:

- Pipette 24 µl premix and put it in PCR tube. Keep them in ice.
- Add 1 µl extracted DNA.
- Centrifuge (by putting PCR tube in open microtube).
- Put it in PCR machine, already programmed (29 cycles + conservation at 4°C).

4. Back Light coloration

e) Centrifuge in micro tubes about 200 µl liquid culture (exposed to UV/temperature + control) 5 min at 15000g. Discard the supernatant.
f) Resuspend in 300 µl NaCl 0,85 % (first washing)
g) Centrifuge again 5 min at 15000g. Discard the supernatant
h) Resuspend in 300 µl NaCl 0,85 %
i) Add 0.2 µl premix AB from Back Light kit
j) Centrifuge 5 min at 15000g. Discard the supernatant
k) Resuspend in 300 µl NaCl 0,85 % (second washing)
l) Let it rest in dark for about 15 minutes

5. Preparing micriscope slides (sterile conditions)

a) Put slides in alcohol and hold them in fire with pliers by inclining slides down not to be burnt Resuspend in 300 µl NaCl 0,85 % (first washing)
b) Pipette 25 µl coloured cultures on slides
c) Put microscope slides at 60°C in Pasteur oven for a few minutes, until drops dry (to prevent bacteria form moving on slides)
d) Observe slides with an epifluorescence microscope